

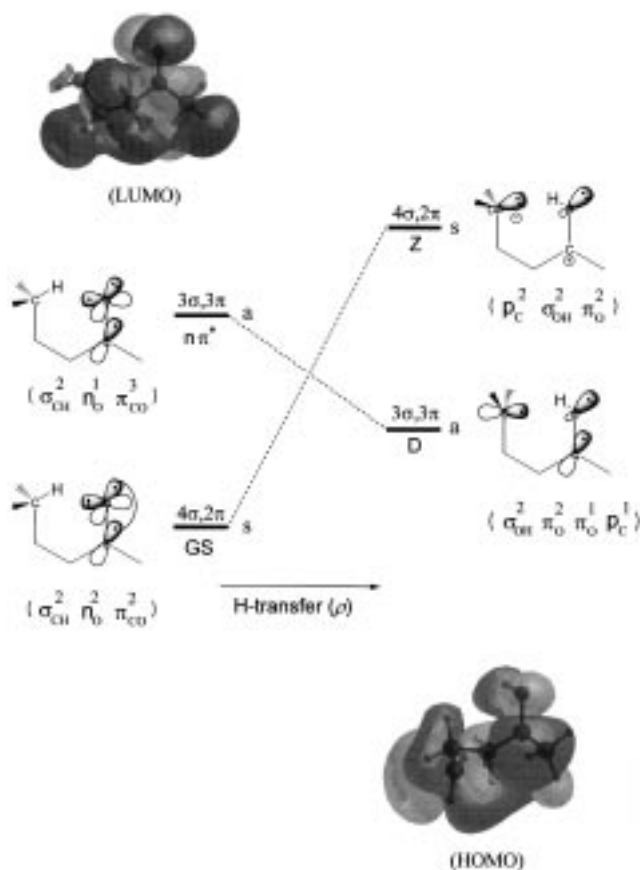


Figure 3. The HOMO and LUMO obtained from our density functional theory calculations, together with simple orbital correlations showing the direct formation of the diradical species. The correlation is with respect to the molecular plane of symmetry. Note that in the optimized geometry calculation of the HOMO and LUMO, this restriction is relaxed as the geometry is not planar.

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An EPR Investigation of the Kinetics of Inclusion of a Persistent Radical in Water-Soluble Calix[4]arenes

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A voluminous literature reporting the synthesis and characterization of calixarenes behaving as highly selective receptors for metal cations and organic guest molecules has appeared during the last twenty years^[1]. These calixarenes are

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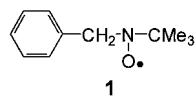
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Supporting information for this article is available on the WWW under <http://www.wiley-vch.de/home/angewandte/> or from the author.

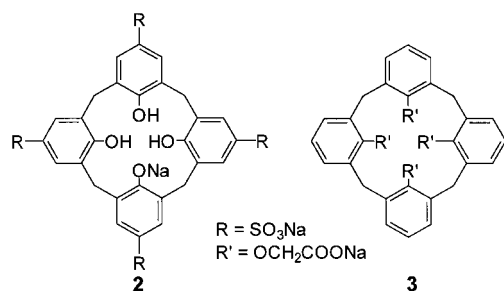
highly selective receptors for metal cations and organic guests. The earliest examples of water-soluble calixarenes were described in the mid 1980s by Ungaro and co-workers^[2] and by Shinkai and co-workers,^[3] while several additional examples have appeared more recently.^[4] The equilibration between host and guest entities in water is a dynamic process taking place at rates higher than the differences in the NMR frequencies. For this reason the experimental NMR spectra appear as averages of those from the free and complexed species, similar to what is observed with inclusion equilibria involving cyclodextrin hosts.^[5]

In contrast to NMR, EPR spectroscopy is characterized by a much faster time scale. When this technique is used to study the complexation of radical species by cyclodextrins (CDs), EPR spectra resulting from the superposition of separate signals from free and complexed species are usually observed.^[6–8] We have recently found that a radical probe particularly suitable for studying inclusion phenomena in aqueous solution by β -CDs is benzyl *tert*-butyl nitroxide (**1**).^[8, 9] The formation of an inclusion complex with this



radical was manifested by large spectral changes arising both from the decrease of nitrogen hyperfine splitting, $a(\text{N})$, induced by the less polar environment of the β -CD host cavity, and from the strongly reduced coupling of the benzylic protons, $a(2\text{H}_\beta)$, due to conformational changes occurring upon complexation. Thus, inclusion into the CD produced a considerable decrease in the overall splitting and significant differences in the resonance fields of the $M_I(2\text{H}_\beta) = \pm 1$ lines for the included and free species. The EPR spectra also showed a strong linewidth dependence on temperature, indicating that the lifetime of **1** in the associated and free form is comparable to the EPR time scale. Analysis of the EPR line shape enabled us to measure the rate constants for the association and dissociation processes.^[9]

Water-soluble calixarenes have been mainly employed to study the inclusion of charged organic species such as tetraalkylammonium cations^[1d, 10] or α -amino acids^[11]. Very little is known on their inclusion properties towards neutral aromatic guest species which, on the other hand, are easily complexed in the solid state.^[1] Here we report the use of benzyl *tert*-butyl nitroxide (**1**) to probe its inclusion in two water-soluble calixarenes—namely, pentasodium 25,26,27,28-tetrahydroxycalix[4]arene-5,11,17,23-tetrakisulfonate (**2**) and tetrasodium 25,26,27,28-tetrakis(carboxymethoxy)-calix[4]arene (**3**).^[12]—and to study the kinetics of the association/dissociation process by analyzing variations in the EPR line shape with temperature.



The EPR spectrum at 298 K of **1**, produced by oxidation of benzyl-*tert*-butylamine ($1.0 \times 10^{-3} \text{ M}$) with oxone ($2\text{KHSO}_5 \cdot \text{KHSO}_4 \cdot \text{K}_2\text{SO}_4$; $4.8 \times 10^{-4} \text{ M}$) in water, is shown in Figure 1a. The spectrum is easily interpreted on the basis of the coupling of the unpaired electron with the nitrogen nucleus and with

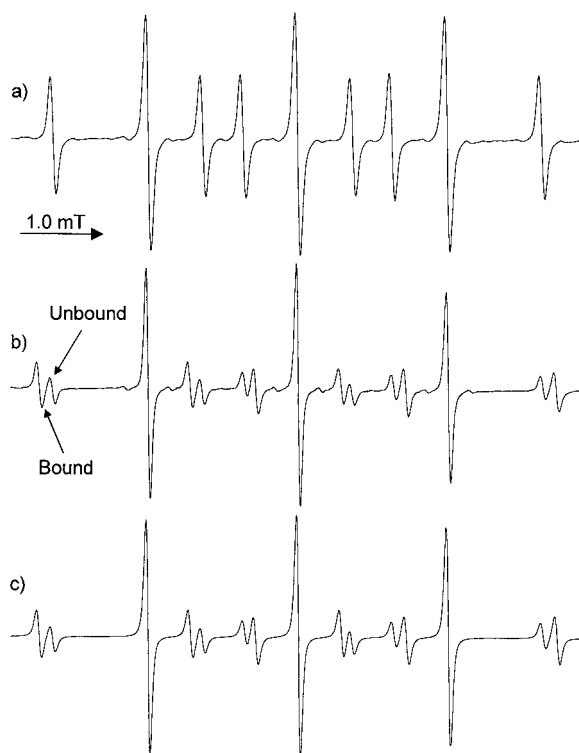
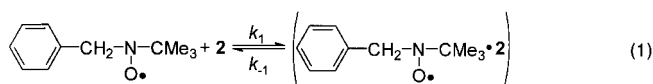


Figure 1. EPR spectra recorded at 294 K of nitroxide **1** ($3 \times 10^{-5} \text{ M}$) in water (a) and in water containing 0.1 M calix[4]arene **2** (b). The simulation (c) of spectrum b was performed by using the following data: free nitroxide (38 %, linewidth 0.023 mT, $a(9\text{H}) = 0.015 \text{ mT}$, $a(\text{N}) = 1.669 \text{ mT}$, $a(2\text{H}) = 1.064 \text{ mT}$, $g = 2.0056$); included nitroxide (62 %, linewidth 0.026 mT, $a(9\text{H}) = 0.015 \text{ mT}$, $a(\text{N}) = 1.686 \text{ mT}$, $a(2\text{H}) = 1.211 \text{ mT}$, $g = 2.0056$); rate constant for association $k_1 = 3.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ and for dissociation $k_{-1} = 2.6 \times 10^6 \text{ s}^{-1}$ [See Eq. (1)].

the two equivalent benzylic protons ($a(\text{N}) = 1.669 \text{ mT}$, $a(2\text{H}) = 1.064 \text{ mT}$, $g = 2.0056$). In the presence of calix[4]arene **2** (0.1 M), additional signals (Figure 1b) assigned to the radical included in the cavity of **2**, which is in equilibrium with the free nitroxide [Eq. (1)], were observed.



When the absolute concentration of **2** was increased, the ratio between included and free species varied linearly and, for values higher than 0.15 M, the spectrum of the complexed radical **1**, characterized by the spectroscopic parameters $a(\text{N}) = 1.686 \text{ mT}$, $a(2\text{H}) = 1.211 \text{ mT}$, and $g = 2.0056$, became dominant. The EPR results were consistent with the formation of 1:1 inclusion complexes of nitroxide **1** with calix[4]arene **2**.

The above-reported data indicate that the hyperfine splitting at nitrogen remains essentially unaffected, while that at

the benzylic protons increases upon inclusion. The similarity of the $a(\text{N})$ values for free and included **1** indicates that the NO group in the complexed radical is exposed to bulk water; it is therefore conceivable that the phenyl moiety is instead selectively bound to the calixarene cavity. On the other hand, the increased splitting of the benzylic proton signals^[13] suggests that inclusion into the calix[4]arene cavity is accompanied by a change either of the preferred conformation adopted by the radical or of the height of the barrier to the internal rotation of the phenyl group.

In the investigated range of temperatures (278–334 K), the EPR spectrum of **1** showed marked selective line-broadening, especially evident on the lines corresponding to $M_I(2\text{H}) = \pm 1$ (Figure 1 b). Such linewidth effects are due to modulation of the proton and, to a smaller extent, nitrogen hyperfine splittings, arising by exchange between the free and included nitroxide.^[9] The rate constants k_1 for the inclusion of **1** by calix[4]arene and k_{-1} for the dissociation of the resulting complex were determined at various temperatures by simulating the exchange-broadened EPR spectra by using well-established procedures^[15] based on the density matrix theory^[16] in the assumption of a two-jump model [see Eq. (1)]. Figure 1 shows, as an example, an experimental spectrum (b) and the corresponding computer simulation (c). The kinetics of the inclusion of **1** by calix[4]arene **2** in water were studied in the temperature range 278–334 K (Figure 2) and the resulting activation parameters $\Delta H^\ddagger$ and $\Delta S^\ddagger$ are reported in Table 1.

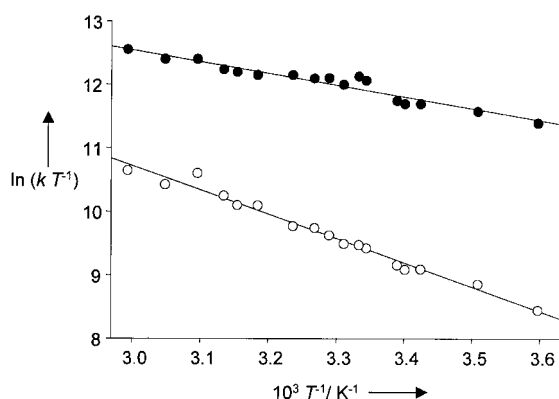


Figure 2. Plot of $\ln(k/T)$ vs. $1000/T$ for the association (●) and dissociation (○) rate constants for inclusion of radical **1** by calix[4]arene **2** in water.

A different behavior was observed when studying the inclusion of nitroxide **1** in calixarene **3**. Although the benzylic proton splittings showed significant variations upon addition of **3** ($a(2\text{H})$ decreased from 1.064 mT in water to 1.030 mT in a 0.14 M solution of **3**), it was not possible to detect separate signals from the free and bound nitroxide. This suggests that, in the present case, the time-averaged spectrum is observed;

this could be due either to fast rates of association and dissociation or to small differences in the spectroscopic parameters of the free and bound species.

An examination of the thermodynamic and kinetic parameters (Table 1) shows that the reaction enthalpy, $\Delta H^\ddagger$, for the inclusion process is negative ($-16.4 \text{ kJ mol}^{-1}$), suggesting that the driving force to complexation is the π – π stabilizing interaction between the phenyl ring of **1** and the aromatic system of the calixarene^[17] or a hydrophobic effect or both. However, the room-temperature value of the affinity constant is small (12.5 M^{-1}) because of the negative entropy of reaction ($-33.9 \text{ J mol}^{-1} \text{ K}^{-1}$) originating from the freezing of the translational degrees of freedom for the guest molecule. It also appears that the difference between the $\Delta S^\ddagger$ for association and ΔS° is small ($-13.4 \text{ J mol}^{-1} \text{ K}^{-1}$), while that between the corresponding enthalpic terms is quite large (31.7 kJ mol^{-1}). This suggests that in the transition state nitroxide and calix[4]arene are already so close that the solvent arrangement around the incipient complex is similar to that of the inclusion compound, rather than that of the separate host and guest molecules. From the large enthalpy of activation, it may be inferred that the phenyl ring of **1** is not yet deep enough inside the calixarene cavity to provide the stabilizing interaction between the guest and host π systems.

In conclusion, water-soluble calixarene hosts form inclusion complexes with the nitroxide radical **1**, the inclusion producing marked differences in the hyperfine splitting constants with respect to the free radical.^[18] The EPR spectra also showed selective line broadening effects due to the exchange between free and included nitroxide, which allowed us to measure, for the first time, the kinetic rate constants and the activation parameters for the inclusion of a neutral molecule in a calixarene host in water.^[19] Even if complementary techniques like UV, NMR, or fluorescence spectroscopy cannot be employed for our probe to check the data, the use of radical **1** for investigating the dynamics of calixarene host–guest interactions and to study structural and medium effects on it, has considerable potential in our view, and should be considered as another method for investigating the kinetics of complexation.^[20] Experiments are currently in progress to check the influence of changing the pH and of adding a cosolvent or inorganic salt on the equilibrium and activation parameters. Moreover, since chiral nitroxides bearing β -hydrogen atoms are readily accessible, the present study can be extended to chiral radical probes.

Experimental Section

Radical **1** was generated by mixing a solution of benzyl-*tert*-butylamine ($4.4 \times 10^{-2} \text{ M}$) in methanol (0.5 mL) and a solution containing oxone (Aldrich; $1.6 \times 10^{-2} \text{ M}$) in water (0.5 mL) with a solution containing variable amounts of calixarene (0.01–0.15 M) in water (20 mL). In all samples the

Table 1. Thermodynamic and kinetic parameters for the inclusion of nitroxide **1** by calix[4]arene **2** in water.

	$\Delta H^\ddagger$ ^[a] [kJ mol ⁻¹]	$\Delta S^\ddagger$ ^[a] [J mol ⁻¹ K ⁻¹]	K^{298} [M ⁻¹]	$\Delta H^\ddagger$ [kJ mol ⁻¹]	$\Delta S^\ddagger$ [J mol ⁻¹ K ⁻¹]	k^{298}
association	-16.4	-33.9	12.5	15.3 ± 2.1 ^[b]	-47.3 ± 7.1	$4.4 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
dissociation				31.7 ± 2.3	-13.4 ± 7.5	$3.5 \times 10^6 \text{ s}^{-1}$

[a] Values derived from the measured activation parameters. [b] Errors correspond to twice standard deviations.

pH was adjusted to 7.4. Samples were then transferred in capillary tubes (1 mm inner diameter) and EPR spectra were recorded using a Bruker ESP300 spectrometer. The instrument settings were as follows: microwave power 5.0 mW, modulation amplitude 0.05 mT, modulation frequency 100 kHz, scan time 180 s. The computed spectra were best-fitted to the experimental ones using a Monte Carlo minimization procedure.^[9, 21] The input data for the minimization program were the number of nonequivalent nuclei, the hyperfine splitting constants of the free and included radical at each temperature, the intrinsic linewidth in the absence of exchange, and the rate constants for the exchange process.

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